Close mapping of the focal non-epidermolytic palmoplantar keratoderma (PPK) locus associated with oesophageal cancer (TOC)

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Focal non-epidermolytic palmoplantar keratoderma (PPK or palmoplantar ectodermal dysplasia type III) is associated with oesophageal cancer in three families: two large pedigrees located in Liverpool, UK and in the midwestern American states and one smaller family from Germany. In these families, the PPK is inherited as autosomal dominant and has a late onset, usually manifesting between 7 and 8 years of age. The disease is characterised by thickening of the pressure areas of the soles, but is not restricted to the feet and also presents with oral leukokeratosis and follicular hyperkeratosis. The disease locus [previously termed the ‘tylosis oesophageal cancer gene’ (TOC) locus] has been mapped to 17q23-qter by linkage analysis. This region is located telomeric to the keratin 16 gene, in which mutations have been identified in focal PPK families who show no increased cancer risk. We describe the close mapping of this locus to the interval between AFMb054zf9 and D17S1603 using haplotype analysis of additional Généthon markers in the region and show that although the American family is unlikely to be related to either of the other two, the UK and German pedigrees may share a common descent. This work provides a basis for positional cloning and candidate gene analysis in order to identify a gene that may be involved in familial oesophageal cancer.

INTRODUCTION

The palmoplantar keratodermas (PPKs) are a complex group of hereditary syndromes, which have been classified clinically according to the presence or absence of epidermolysis and the pattern of hyperkeratosis on the palms and soles. Diffuse, punctate and focal forms of PPK have all been described (1,2). In this study, we have focused on three pedigrees, two extensive families from Liverpool, UK and the midwestern American states and a third, smaller, family from Germany, in which focal non-epidermolytic PPK is associated with a high risk of developing oesophageal cancer. The PPK observed in the Liverpool family (‘tylosis’) was originally defined as being of the diffuse type, but a dermatological re-examination of members of this family who are affected with the skin disorder has indicated that the distribution of the keratoderma may be better described as focal PPK. In a recent reclassification of the PPKs, focal PPK associated with oesophageal cancer has been termed palmoplantar ectodermal dysplasia type III (2).

The association between focal PPK (‘tylosis’) and oesophageal carcinoma in a group of related patients in Liverpool, UK was initially reported in 1958 by Howell-Evans (3), (McKusick ref. 148500), and has recently been shown to comprise a single family containing 345 individuals, 92 of whom have focal PPK (4). Subsequently, a large Midwestern American family containing 125 affected individuals with an identical pattern of PPK was described, in which the skin disorder was associated with oesophageal and other cancers (2). In both of these families, the PPK is inherited as autosomal dominant with complete penetrance (onset at age 7–8 years) and presents with associated oral leukokeratosis (synonymous with oral hyperkeratosis) and follicular hyperkeratosis (2,4). The disease is clinically similar to focal PPKs in which there is no increased risk of malignancy, apart from the absence of nail involvement. A number of reports have also described the association of both the diffuse and punctate forms of PPK with internal neoplasia (5–8).

Of the UK family, 92 have been diagnosed as having focal PPK of which 32 have died, 21 of cancer of the oesophagus (4). In this pedigree, there have been no oesophageal cancers in family

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RESULTS

Haplotype analysis

Ten proximal and five distal recombination events within the 6 cM region between D17S929 and D17S937 localized the focal PPK locus in the UK and US families between AFMb054zf9 and D17S1603 (Figs 1 and 2).

In addition, a number of cross-over events were observed between markers which could not be separated on a previous Généthon map (Weissenbach, unpublished), which enabled us to refine the order of some of the DNA marker loci in this region (Fig. 3).

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Figure 2. Representative cross-over events in the UK (a) and US (b) pedigrees showing localization of the TOC locus. (■●) Individual with focal PPK and malignancy, oe=oesophageal, ut=uterine cancer. (☐☐) Individual with focal PPK but no malignancy. (□□) Individual with no focal PPK or oesophageal cancer (unaffected). Order of markers: (a) D17S929, D17S1602, AFMc008we1, AFMb054zf9, D17S801, D17S1603, D17S785, AFMa312ya5, AFMa134wa9, D17S937. (b) D17S929, D17S1602, AFMc008we1, AFMb054zf9, D17S801, D17S1603, D17S785. Allele numbers were assigned independently by the two main researchers and do not correlate between the families.

that the US family is descended from a distinct ancestral mutation to that observed in the UK and German families, and that both mutations produce similar focal PPK phenotypes.

Table 1. Comparison of pedigrees

<table>
<thead>
<tr>
<th>DNA marker</th>
<th>PPK pedigrees</th>
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<tbody>
<tr>
<td></td>
<td>USA</td>
</tr>
<tr>
<td>AFMc008we1</td>
<td>120</td>
</tr>
<tr>
<td>AFMb054zf9</td>
<td>255</td>
</tr>
<tr>
<td>D17S801</td>
<td>278</td>
</tr>
<tr>
<td>D17S1603</td>
<td>229</td>
</tr>
<tr>
<td>D17S785</td>
<td>209</td>
</tr>
</tbody>
</table>

Numbers in the body of the table refer to the size (in base pairs) of the allele found in the disease haplotype in the appropriate family.

DISCUSSION

In this paper, we have investigated three apparently unrelated families with a similar skin phenotype that is associated with an increased risk of oesophageal cancer (2,4,10). The two phenotypes segregate together in all three pedigrees, two of which are extensive (6–7 generations with >100 members with the skin phenotype), thus implying that it is the same gene that causes the skin disorder and oesophageal cancer in these individuals. This conclusion is strengthened by the observation that two distinct microsatellite marker haplotypes are tightly linked to the disease gene in the three families.

We have now demonstrated a reduction of the genetic interval containing the focal PPK/oesophageal cancer (TOC) locus to approximately 1 cM between the DNA marker loci AF Mb054zf9 and D17S1603 and have revised the locus order originally proposed by Généthon. The unaffected individual, VI:83, who defines the proximal limit has been carefully re-evaluated by the dermatologists involved in this study to ensure that no indications of focal PPK could be observed. A genomic region of this order of magnitude is particularly amenable to gene mapping by positional cloning techniques and we are currently mapping the physical region between AF Mb054zf9 and D17S1603 using YAC, PAC, BAC and cosmid libraries.

There is no evidence to date that demonstrates an association between allelic imbalance (or loss of heterozygosity) in this region and sporadic oesophageal or head and neck cancers (11–14). However, frequent loss of heterozygosity in oesophageal carcinomas has been described in the region containing the BRCA1 locus located 30–40 cM centromeric to the putative focal PPK/oesophageal cancer (TOC) locus (15). Current projects being undertaken by our groups include the investigation of allelic imbalance in sporadic oesophageal cancers using microsatellite marker loci located within the TOC minimal region.

It is of interest that, whilst the lineage of the US family may be traced back to individuals in Germany with focal PPK lesions, the US and German pedigrees studied here appear to be genetically unrelated at the disease locus. In contrast, there is no known genealogical link between the UK and German families and their respective incidences of oesophageal cancer are different, yet they appear to share a haplotype in the region of the disease locus. However, the alleles shared by these two families...
at the AFMb054zf9 and D17S801 loci are the most common in the general population (55% and 30%, respectively; data from the Genome DataBase and unpublished) and segregate with both the affected and the unaffected chromosome. Thus, the UK and German families may not be related, but carry distinct mutations on a common haplotype. However, as these families share a less common allele at the D17S1603 locus (12% in the general population; data from the GDB), the hypothesis of their common descent is the more likely alternative.

This work therefore provides a basis from which transcript mapping and gene identification can proceed. The isolation of the familial focal PPK/oesophageal cancer gene (TOC) will also allow the investigation of a possible role for this gene in sporadic oesophageal cancer.

MATERIALS AND METHODS

PCR analysis

A total of 79 patients from the UK, 45 from the US, and five from the German families were genotyped using 11 highly polymorphic Généthon microsatellite markers localized to the previously defined interval of 6 cM on the long arm of chromosome 17 (AFMa134wa9, AFMa312ya5, AFMb054zf9, AFMc008we1, D17S785, D17S801, D17S929, D17S937, D17S939, D17S1602, D17S1603). Typing was performed on genomic DNA in a standard 25 µl PCR reaction at annealing temperatures of between 52 and 62°C depending upon the primer pair under investigation. PCR products were then analyzed, either on 7–10% non denaturing polyacrylamide gels and visualized by the silver staining method of Gottlieb & Chavko (16), or by using an ABI automatic sequencer and Genotyper software.

Haplotype analysis

Haplotypes were formed using the alleles from the 11 microsatellite markers and genetic recombinations identified. In meioses where phase could not be determined unequivocally, the haplotypes showing the minimal number of recombination events were constructed. These were then used to refine the published genetic map and to determine a minimal region containing the focal PPK/oesophageal cancer gene (TOC).

ACKNOWLEDGEMENTS

This work was supported in part by the North West Cancer Research Fund (UK) (JMR); the Imperial Cancer Research Fund (UK) (JMR); the Imperial Cancer Research Fund (UK) (JMR); the Imperial Cancer Research Fund (UK) (JMR); the Imperial Cancer Research Fund (UK) (JMR); the Imperial Cancer Research Fund (UK) (JMR); the Imperial Cancer Research Fund (UK) (JMR). This work was supported in part by the North West Cancer Research Fund (UK). The following Généthon markers have recently been assigned D17S1790. The following Généthon markers have recently been assigned D17S1839; AFMa312ya5 - D17S1817; AFMa134wa9 - D17S1864; AFMb054zf9 - D17S1860; AFMc008we1 - D17S1862, D17S1602, D17S1603. Typing was performed on genomic DNA in a standard 25 µl PCR reaction at annealing temperatures of between 52 and 62°C depending upon the primer pair under investigation. PCR products were then analyzed, either on 7–10% non denaturing polyacrylamide gels and visualized by the silver staining method of Gottlieb & Chavko (16), or by using an ABI automatic sequencer and Genotyper software.

REFERENCES


NOTE ADDED IN PROOF

The following Généthon markers have recently been assigned D segment numbers: AFMc008we1 - D17S1864; AFMb054zf9 - D17S1860; AFMa312ya5 - D17S1862, D17S1602, D17S1603. Typing was performed on genomic DNA in a standard 25 µl PCR reaction at annealing temperatures of between 52 and 62°C depending upon the primer pair under investigation. PCR products were then analyzed, either on 7–10% non denaturing polyacrylamide gels and visualized by the silver staining method of Gottlieb & Chavko (16), or by using an ABI automatic sequencer and Genotyper software.

ABBREVIATIONS

PPK, palmoplantar keratoderma; TOC, tylosis oesophageal cancer; BRCA1, breast cancer gene 1; cM, centiMorgans; YAC, yeast artificial chromosome; BAC, bacterial artificial chromosome; PAC, P1 artificial chromosome.